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ABSTRACT:

The oyster toadfish (Opsanus tau) is an ideal model to examine the effects of anthropogenic noise on behavior because they rely on acoustic signals for mate attraction and social interactions. We predict that oyster toadfish have acclimated to living in noise-rich environments because they are common in waterways of urban areas, like New York City (NYC). We used passive acoustic monitoring at two locations to see if calling behavior patterns are altered in areas of typically high boat traffic versus low boat traffic (Pier 40, NYC, NY, and Eel Pond, Woods Hole, MA, respectively). We hypothesized that toadfish in NYC would adjust their circadian calling behavior in response to daily anthropogenic noise patterns. We quantified toadfish calls and ship noise over three 24-h periods in the summer reproductive period at both locations. We observed an inverse relationship between the duration of noise and the number of toadfish calls at Pier 40 in comparison to Eel Pond. Additionally, toadfish at Pier 40 showed significant differences in peak calling behavior compared to Eel Pond. Therefore, oyster toadfish may have acclimated to living in an urban environment by potentially altering their communication behavior in the presence of boat noise. © 2024 Acoustical Society of America. https://doi.org/10.1121/10.0024763

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I. INTRODUCTION

Many species of fishes use sound to communicate (Ladich, 2013; Rice et al., 2022), detect prey and predators (Remage-Healey et al., 2006), navigate and select habitats (Stanley et al., 2012), and learn about their environment (Slabbekoorn and Bouton, 2008). Shipping and seismic exploration can cause intense anthropogenic noise that propagates long distances (Martin and Popper, 2016), and can potentially mask or block necessary signals. Noise from vessels typically includes frequencies below 1000 Hz (Popper, 2003), can travel hundreds of kilometers, and also increases ambient sound levels in the ocean (Hildebrand, 2009). Most fish typically hear best within the frequency band of 30–1000 Hz and anthropogenic noises are within the hearing ranges of many fishes and marine mammals (Popper, 2003; Slabbekoorn et al., 2010). When noise is within the biological range of acoustic communication, it can mask and inhibit the detection or discrimination of salient stimuli (Fay and Simmons, 1999), and could ultimately affect an animal's

a) This paper is part of a special issue on Fish Bioacoustics: Hearing and

ability to survive (Slabbekoorn et al., 2010). Anthropogenic noise levels in oceans and other waterways have increased in recent decades (Hildebrand, 2009), due primarily to the large numbers of smaller pleasure craft, commercial fishing boats, and large vessels (Andrew et al., 2011). Many reviews have extensively assessed the negative impacts of sound on the behavior and physiology of fishes (Slabbekoorn et al., 2010; Radford et al., 2014; Sabet et al., 2016; de Jong et al., 2020). Fishes that rely on sound for reproduction or survival may be the most affected by the increasing noise levels in our waterways (Frisk, 2012; Radford et al., 2014). Yet there are limited studies on how ecologically relevant sounds affect fishes (Mackiewicz et al., 2021; Ricci et al., 2017), and even fewer studies on the effect of noise on animals that require sound for reproductive-related communication.

Based on their abundance in New York City waters

ceases in August (Fine, 1978). Male oyster toadfish use a

multi-harmonic acoustic call to attract females called a

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[[]Hom and Forlano, 2023; Fig. 1(A)], oyster toadfish (Opsanus tau, family Batrachoididae) present an excellent model to examine behavioral adaptations for living and thriving in a noisy environment. Oyster toadfish exhibit seasonal and daily variation in calling behavior, and their reproductive season is typically between May and July, and

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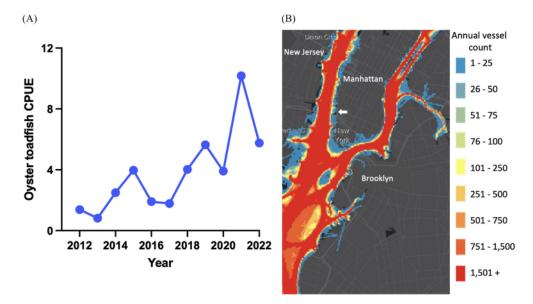


FIG. 1. (Color online) Reproduced from Hom, K. N., and Forlano, P. M. (2023). "Dopamine in the auditory system of vocal toadfishes: Potential adaptation for noisy aquatic environments," in *The Effects of Noise on Aquatic Life Popper*, edited by A. N. Popper and A. Hawkins (Springer, New York) https://doi.org/10.1007/978-3-031-10417-6, with permission of Springer Nature. (A) Data from un-baited crab traps set at Hudson River Park (Pier 26 and Pier 40, New York, NY, yellow and white arrows, respectively). Traps were checked regularly to monitor the natural population of fishes within the Hudson River system. The catch per unit effort (CPUE) was calculated for oyster toadfish populations. (B) Automatic identification system (AIS) tracking information was collected from commercial vessels and large recreational boats. The map shows boat traffic within New York and New Jersey waters from 2021.

"boatwhistle" (Fine, 1978; Gray and Winn, 1961) with dominant frequencies between 90 and 200 Hz (Fine, 1978) that correlate positively with water temperature (Tavolga, 1958; Winn, 1972; Edds-Walton *et al.*, 2002; Maruska and Mensinger, 2009). Male advertisement calls can be a signal of male quality and a determinant of later reproductive success (Winn, 1972; Balebail and Sisneros, 2022; Amorim *et al.*, 2010; Vasconcelos *et al.*, 2012; Amorim *et al.*, 2016). Boatwhistle duration ranges between 200 and 650 ms (Tavolga, 1958; Edds-Walton *et al.*, 2002; Mensinger, 2013) and males make calls throughout the day. Male calling behavior is characterized by infrequent calls during midday, an increase in calling at sunset, a peak of calling between 1900 h and 0200 h with a tapering off after sunrise (Fine, 1977; Ricci *et al.*, 2017; Van Wert and Mensinger, 2019).

Multiple species of toadfishes (Opsanus tau, Opsanus beta, Halobatrachus didactylus) have been used as a model to study the effects of anthropogenic noise on hearing sensitivity and behavior. These studies have found that exposure to boat noise decreased communication distance, interfered with male-to-male interactions, decreased calling rate and reproductive success, decreased hearing sensitivity, and increased levels of stress (Alves et al., 2016; Krahforst et al., 2016; Luczkovich et al., 2016; Rogers et al., 2020; Cartolano et al., 2020; Alves et al., 2021; Amorim et al., 2022). However, these studies played recorded boat noise with a speaker rather than observing the behavior of toadfish reacting to the boat noise they are exposed to in their environment. A recent study using Opsanus tau measured the effects of in situ boat noise on calling behavior and found that toadfish reduce calling behavior after exposure to boat noise (Mackiewicz et al., 2021). However, the animals were in a relatively quiet soundscape with little boat traffic. Thus,

there is a need to examine the behavior of animals in soundscapes where there is near constant exposure to human generated noise.

While oyster toadfish are found along the eastern seaboard of the United States (Gudger, 1910), it is notable they are abundant in waterways surrounding the largest urban center in the country like New York City (NYC), NY (Anderson et al., 2008). Despite living in noisy soundscapes, toadfish populations alongside NYC (Hudson River, New York, NY) have maintained and potentially increased population size in the last 10 years [Hom and Forlano, 2023; see Fig. 1(A)]. While other animals might avoid aversive stimuli (Popper, 2003), oyster toadfish establish nests, are responsible for parental care, and males maintain high nest fidelity (Gray and Winn, 1961; Maruska and Mensinger, 2009; Mensinger, 2013). Therefore, oyster toadfish might be more susceptible to the detrimental effects of noise since they do not evacuate the area when noise is present (Faulkner et al., 2018). We predict that toadfish populations might be altering their call patterns or behavior in the presence of noise as an acclimation for communicating effectively in urban aquatic environments.

Passive acoustic monitoring (PAM) is a noninvasive way to detect and localize soniferous animals (Wall et al., 2013; Ricci et al., 2017; Mackiewicz et al., 2021; Putland et al., 2018; Rountree et al., 2006). A previous study (Anderson et al., 2008) monitored the Hudson River using PAM; however, this study focused on documenting soniferous species, primarily in the evening because that is when they predicted that fish would call the most and to avoid potential noise from boats and therefore did not monitor changes in behavior over the course of a day. Similar PAM recordings of oyster toadfish behavior have been made in

1231

Eel Pond, Woods Hole, MA (Putland et al., 2018). Eel Pond is a secluded bay with minimal activity of large vessels and is regulated by a draw bridge; unlike the Hudson River, where there is recreational and commercial activity throughout the day.

The goal of the present study was to (1) use PAM to identify boat activity and boats that produce noise at Pier 40 (Hudson River, NY, NY), (2) use PAM to examine any differences in calling behavior patterns of oyster toadfish in a noisy environment (Pier 40) to that of a quiet environment (Eel Pond, MA) over a 24-h period, and (3) assess if the toadfish in the noisy environment are changing their calling behavior in response to acute noise in their environment. We hypothesized there would be significantly more noise present at Pier 40 than at Eel Pond and that calling behavior would decrease or change in the presence of noise, regardless of location.

II. METHODS

A. Boat activity of the Hudson River

Boat activity was measured at Pier 40, Hudson River, New York, NY (Fig. 2, 40.73° N, 74.01° W) on August 11, 2022. We placed a hydrophone (H2dM, Aquarian Audio & Scientific, Anacortes, WA; sensitivity unknown) in the Hudson at a depth of 5 m. A GoPro (GoPro, San Mateo, CA) was placed on the pier facing the Hudson River, and every boat that crossed the camera's view of the river was documented and assessed as either commercial or recreational. Measurements were taken for 7h between 10:00h and 17:00 h. Thirty-two minutes of the visual and acoustic recording were merged using iMovie (Apple, Cupertino, CA) to identify the sounds of specific boats and the number of boats crossing within that period.

B. Passive Acoustic Monitoring (PAM)

PAM was conducted at Pier 40 (Fig. 2, 40.73° N, 74.01° W) in June of 2022. Three days (June 6th–9th) were selected for analysis because it was the peak of the breeding season for oyster toadfish. A hydrophone with an integral digital recorder (ST300 STD, Ocean Instruments, New Zealand; sensitivity of $-175.9 \, dB$ re. $1 \, V/\mu Pa$ at max gain; frequency response from 20 Hz to 60 kHz; 24 kHz sampling rate) was secured 1 m above the river floor and set to record 40 min per hour, continuously for three days. Depth at Pier 40 varies between 3.3 and 5.4 m depending on tide and moon phase. A total of 73h of acoustic recordings were collected at this location. Hourly water temperature was on average 17.55 °C ($\pm = 0.347$ °C, standard deviation) as recorded by the SoundTrap. PAM was simultaneously conducted in the Eel Pond (Fig. 2, 41.53° N, 70.67° W) on June 6th for temporal overlap with Pier 40. Two additional days of recordings at Eel Pond (May 27th and 28th) were matched for temperature (17 °C) with Pier 40. At Eel Pond, a High Tech HTI-96-min hydrophone, (High Tech Inc.) Long Beach, MS; sensitivity of $-164.9 \,\mathrm{dB}$ re. $1 \,\mathrm{V}/\mu\mathrm{Pa}$ at max gain; frequency response of 2 Hz to 30 kHz) was used with a SoundTrap ST4300 digital acoustic recorder (Ocean Instruments, New Zealand, 4dB gain; 24kHz sampling rate). The hydrophone was beneath the research dock, and suspended 1 m above the substrate. Depth at Eel Pond varies between 2.4 m and 3.4 m depending on tide and moon phase.

Despite temperature matching days between Pier 40 and Eel Pond, the two days in May at Eel Pond had far fewer calls than the June days at either location. All days could be compared for daily calling patterns by using the percent daily calls per hour as a metric (see the following).

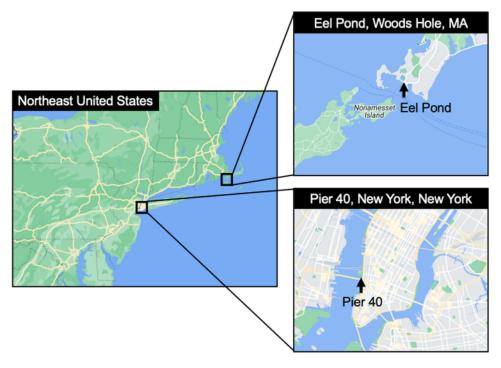


FIG. 2. (Color online) The two test locations in this study: Pier 40, New York City, NY, and Eel Pond, Woods Hole, MA. The left map is of the Northeast United States. Top right graph is of Eel Pond and the bottom right graph is of Pier 40 on the Hudson River (indicated by arrowheads, respectively).

C. Identifying boatwhistles and noise over 24 h

For most analyses, two-minute samples from the beginning of each hour were examined. Sound files were viewed in Raven Pro 1.6 (Cornell Lab of Ornithology, Ithaca, NY), and individual toadfish calls and vessel noise events were manually identified and tracked in selection tables. The number of calls and seconds of noise per sample were recorded. Five observers identified calls and noise. These individuals were trained together and blindly examined select files to ensure consistent identifications. Any given day was examined by only one researcher and the before, during, and after noise analysis (see the following) was analyzed by one observer.

D. Call pattern comparison between sites

To evaluate overall calling behavior between locations, we compared the average number of calls per day between Eel Pond and Pier 40 using a non-parametric Mann-Whitney unpaired two tailed t-test. To evaluate overall noise differences between locations, we compared the average seconds of noise between locations using a non-parametric unpaired two-tailed Mann-Whitney test. Analyses were calculated in GraphPad Prism.

To compare the pattern of daily calling between Pier 40 and Eel Pond, calls were normalized by dividing the number of calls identified in each 2 min period, by the total number of calls found that day, then binned into four periods, inclusive: Moming (from midnight to 5 AM), Day (from 6 AM to 11 AM), Afternoon (from noon to 5 PM), and Night (from 6 PM to 11 PM). The proportion of total daily calls occurring in each bin was calculated and compared between sites. A linear model analysis of variance (ANOVA, type III) was used to examine the impact of location, period, and the interaction of location and period on proportion of daily calls. Residual plots and Q-Q plots were examined to ensure the model met the required assumptions. Analysis was done in R (RStudio Team, 2023).

E. Noise comparisons

Ambient sound was compared between Pier 40 and Eel Pond. To calculate ambient sound, 40 samples (\bar{x} length-= 1.86, \pm = 0.47 s, indicating mean and standard deviation throughout) were taken between 11 AM and 1 PM at each location. At Pier 40, ambient samples averaged 1.86 + 0.47 s (standard deviation), while at Eel Pond they averaged 1.78 + 0.31 s. Samples were taken when neither boat noise nor advertisement calls were detected but were otherwise randomly selected. Ambient samples were bandpass filtered (inverse Fourier transform, Hanning window; ffilter function in R library seewave) to remove sound below 20 Hz, the higher low frequency range of the two hydrophones, and above 800 Hz, the upper limit of the toadfish hearing range (Yan et al., 2000). The getRMS function (window length 24 000 samples, overlap of 50%; R library soundgen) was used to calculate root mean square (RMS) amplitude, and the sensitivities of the hydrophones were used to calculate SPL as dB re 1 μ Pa. GraphPad Prism was used to test for differences.

To determine if acute noise events were in the hearing range of the toadfish, 40 additional samples ($\bar{x}=1.91$ $\pm=0.35$ s, Pier 40; $\bar{x}=1.96$ $\pm=0.50$ s, Eel Pond) were taken during noise events between 11 AM and 1 PM at each location. Noise samples were also bandpass filtered between 20 and 800 Hz (Yan *et al.*, 2000) and getRMS was again used to calculate sound pressure level (SPL). SPL for noise vs ambient was then compared, and ambient samples were compared between locations using a non-parametric Mann Whitney unpaired two-tailed test on GraphPad Prism.

Power spectra for the previously noted ambient sound and acute noise events were calculated using the meanspec function (R library seewave) with Hanning window, length of 24 000 samples, and overlap of 50%. These spectra were bandpass filtered from 20 Hz to 12 kHz, to give a more complete sense of the sound. Individual spectra were averaged at each location and plotted in GraphPad Prism.

F. Call rate and vessel noise: Before, during, after analysis

To examine the effects of vessel noise on call rate, two analyses were conducted for samples from Pier 40. Second, calls were examined before, during, and after discrete noise events during nighttime hours (22:00 h and 04:00 h, June 6th-9th). Sound files at other times of the day were largely continuous with noise and therefore an analysis of this type could not be conducted. Additionally, we chose files with a single boat noise event to assess changes in behavior due to single noise events (excluding other potential noise events). These analyses could not be conducted at Eel Pond because there were not sufficient noise events in the recordings. A one-way repeated measures ANOVA was conducted to compare the effect of noise on the number of calls before, during, and after noise. To examine call change percent, the calculation from Mackiewicz et al. (2021) was used for this study [see Eq. (1)]. Analyses were done using GraphPad Prism:

$$= \left(\frac{Number\ of\ Calls\ Post\ Exposure}{Number\ of\ Calls\ Pre\ Exposure}*100\right) - 100.$$
(1)

III. RESULTS

A. PAM and boat activity of the Hudson River

A total of 245 boats (commercial and recreational) were identified over a 7-h period, with an average of 35 boats per hour [± 8.124 , Fig. 3(A), red triangles] ranging between 25 and 48 boats. We identified more commercial vessels [$\bar{x} = 25.14 \pm 5.58$, Fig. 3(A), blue circles] in comparison to recreational vessels [$\bar{x} = 9.8 \pm 3.38$ Fig. 3(A), magenta squares]. We observed barges, ferries, government vessels,

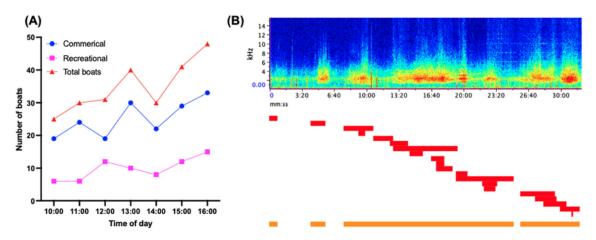


FIG. 3. (Color online) Boat activity of the Hudson River. (A) Over a 7-h period, boats were counted that crossed Pier 40. Recreational boats (magenta, squares), commercial vessels (blue, circles), and the total number of boats (red, triangles) were counted between 10:00 h to 17:00 h. (B) A sample of visual boat counts with simultaneous audio is shown. A spectrogram of boat noise from Pier 40 was aligned with a video recording from a GoPro. Individual boat occurrences were denoted with red rectangles based on the video analysis, and the orange rectangles represent the total time boats were present. Twenty boats passed in a period of 32-min and encompassed 81.4% of the sample.

tour ships, high speed pleasure boats, fishing boats, sailboats, and jet skis.

A sample of a 32-min period is shown in Fig. 3(B). A total of 20 boats (red rectangles) were identified visually and aligned with the corresponding spectrogram (frequency in kHz over time). Frequencies ranged from 0.48 to 15 kHz, with the most power of the noise in the 4–5 kHz range. Over the course of the 32 min, multiple boats were present at the same time, creating an overlapping section of noise. The total time where boats were present or overlapped, encompassed 81.4% of the 32-min sample.

B. Calling behavior at Pier 40 and Eel Pond over 24 h

At Pier 40, we counted a total of 3658 calls and $1035 \,\mathrm{s}$ of noise in our three days of sampling for 2 min in each hour, an average of 50.11 calls (\pm 33.59) and 36.07 s (\pm 48.57 s) of noise (Fig. 4). The mean number of calls and

proportion of noise were averaged for each hour and plotted against each other [Fig. 4(A)]. A simple linear regression was calculated between numbers of calls per hour and proportion of noise [Fig. 4(B)]. A significant inverse relationship between the proportion of noise and the number of calls in an hour was found [F(1, 71) = 8.045, p = 0.00808 with an R^2 of 0.092; Fig. 4(B)].

At Eel Pond, we counted a total of 1727 calls and 985 s of noise in our 2-min segments/h encompassing three days of recordings. Mean number of calls and proportion of noise were identified for each hour [Fig. 5(A)]. A simple linear regression was calculated between the number of calls per hour and the proportion of noise [Fig. 5(B)], and no correlation was identified [F(1, 70) = 1.472, p = 0.2291 with an R^2 of 0.0206]. Using these data, we identified an average of 69 (\pm 37.79) calls and 13.69 s (\pm 34.69 s) of noise (Eel Pond, Fig. 6). There was a significant difference in the average number of calls between Eel Pond and Pier 40 U = 600.5,

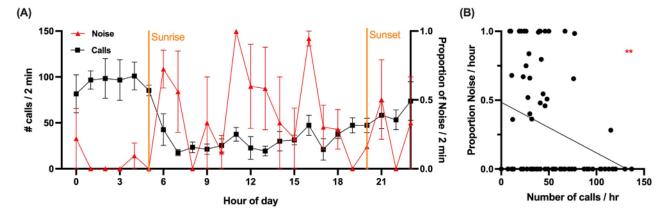


FIG. 4. (Color online) Calling behavior and proportion of noise over three days at Pier 40. (A) Calls were counted over a 2 min period every hour of a 24-h period. Mean number of calls for each hour (black squares) with standard deviation (bars). The amount of noise in each corresponding 2-min segment was identified (red triangles) and divided over 120 s to create a proportion of noise in each hour. Sunrise and sunset are indicated by orange lines. There is an overall inverse relationship between calling and noise. (B) The number of calls counted and the corresponding proportion of noise were compared using a linear regression (** = p = 0.0088).

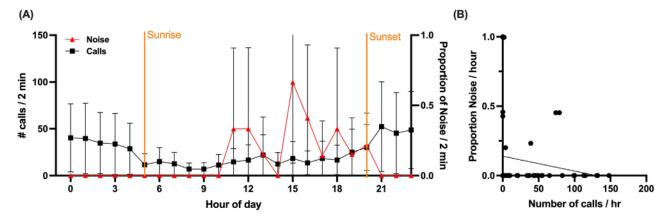


FIG. 5. (Color online) Calling behavior and proportion of noise over three days at Eel Pond. (A) Calls counted over a 2-min period (black squares) in each hour are graphed against time. The daily calling patterns match what has been previously reported (Fine, 1977; Ricci et al., 2017; Van Wert and Mensinger, 2019). The proportion of noise identified in the same 2 min period is shown in red triangles. The orange lines denote sunset and sunrise. (B) There was no significant correlation between the number of calls per hour and the proportion of noise per hour.

p = < 0.0207 [Fig. 6(A)]. We also compared the average seconds of noise at each location and found a significant difference of U = 1936, p = 0.00095 [Fig. 6(B)].

To compare toadfish calling behavior between our locations, we compared the average proportion of calls per hour [Fig. 7(A)] and per period over a 24-h day [Fig. 7(B)]. Eel Pond [Fig. 7(B), gray] exhibited the highest mean number of calls at night ($\bar{x} = 51.88$) in comparison to Morning ($\bar{x} = 29.84$), Day ($\bar{x} = 7.42$), and Afternoon ($\bar{x} = 10.86$). Pier 40 [Fig. 7(B), blue] exhibited the highest mean number of calls in the Morning ($\bar{x} = 45.79$) in comparison to Day ($\bar{x} = 13.08$), Noon ($\bar{x} = 14.14$), and Night ($\bar{x} = 26.22$). The proportion of daily calls falling into each period was examined using a linear model with type III ANOVA. Periods, locations, and the interaction of period and location were compared [Fig. 7(B)]. The interaction of period and location was significant [F(3, 16) = 11.34; p < 0.001]. A post hoc test with false discovery rate (FDR) correction showed a

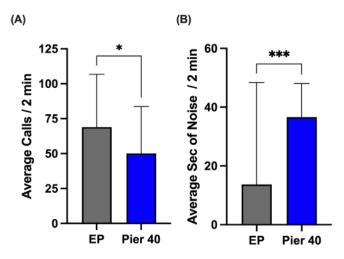


FIG. 6. (Color online) Calling behavior of oyster toadfish at different locations. (A) We compared the number of calls calculated in two minutes at both of our locations: Eel Pond (EP, gray bars) and Pier 40 (blue bars). There were significantly more calls counted at Eel Pond than Pier 40 (*=p<0.0207). (B) We also found a significant difference in average seconds of noise between EP and Pier 40 (***p<0.00095).

significant difference at Night (hours 18–23) between Pier 40 and Eel Pond (p < 0.005), where Pier 40 had a lower proportion of calls. Morning (hours 0–5) was also significantly different between locations (p = 0.015), where Pier 40 had a higher proportion of calls.

We examined samples of ambient sound and boat noise at each location [Eel Pond, Fig. 8(A); Pier 40, Fig. 8(C)]. We compared ambient sound and boat noise that fell within the hearing range of oyster toadfish (60–800 Hz; Yan et al., 2000) for each location [Eel Pond, Fig. 8(B); Pier 40 Fig. 8(D)]. At Eel Pond, the dB SPL of the ambient sound ranged from 92.78 to 114.7 dB SPL with a mean of 98.96 (± 4.584), while the dB SPL of the boat noise ranged from 97.05 to 118.5 with a mean of 106.3 (\pm 6.298). The amplitude (dB SPL) of the boat noise was significantly higher than ambient sound at Eel Pond, U = 52, p < 0.0001 [Fig. 8(B)]. At Pier 40, the dB SPL of the ambient ranged from 104.1 to 125.6 with a mean of 114.6 (± 4.282) and the dB SPL of the boat noise ranged from 110.5 to 127.7 with a mean of 119.2 (\pm 3.541). Boat noise was significantly higher than ambient sound, U=38. p < 0.0001 [Fig. 8(D)]. We compared the ambient sound levels at each location and found that the ambient sound at Pier 40 was significantly higher than the ambient sound at Eel Pond, U=0, p<0.0001. Finally, we compared the vessel noise levels at each location, and vessel noise was significantly higher than the vessel noise at Eel Pond, U=0, p<0.0001.

C. Before, during, and after noise analyses at Pier 40

Calls were measured before, during, and after 13 discrete noise events [Fig. 9(A)]. The average length of a noise event was 148 s (\pm 60.36 s). We computed the average number of calls before ($\bar{x} = 34.19 \pm 13.27$), during ($\bar{x} = 34.0 \pm 12.10$), and after exposure ($\bar{x} = 31.27 \pm 13.22$). A oneway repeated measures ANOVA was conducted to compare the effect of noise on number of calls before, during, and after noise. There was no significant effect of noise on call behavior [F(2, 12) = 16.79, p = 0.30].

The call change percent showed a normal distribution, and the call range varied between +34.61 and -49.12%

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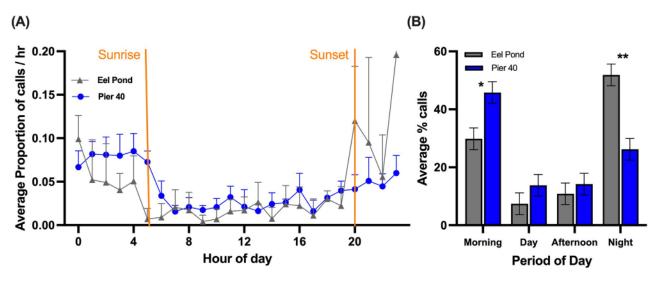


FIG. 7. (Color online) Calling behavior between Pier 40 and Eel Pond over a 24-h day. (A) The average proportion of calls was identified for each hour at each location: Pier 40 (blue circles) and Eel Pond (EP, gray triangles). Sunrise and sunset are designated by an orange line. (B) The average percent of calls was calculated for each hour and binned in four equal periods of a day: Morning, Day, Afternoon, and Night. There was a significant difference between location and period (p < 0.001). A post hoc test reveals significant differences in average call percent between locations in the morning (p < 0.05) and in the night (p < 0.005).

[Fig. 9(B)]. The average call change percent was below zero (average=-7.17, \pm 24.89), indicating an overall but non-significant decrease in calling behavior after exposure.

IV. DISCUSSION

Here, we characterized the daily vocal courtship behavior of the oyster toadfish in relation to anthropogenic noise in an urban aquatic environment (New York, NY) with high

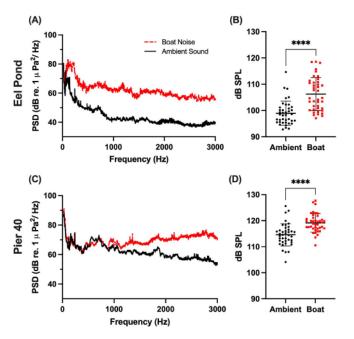


FIG. 8. (Color online) Power spectrum of ambient sound and boat noise at each location. Average power spectra (dB re. 1 μ Pa²/ Hz) for Eel Pond (A) and Pier 40 (C) comparing samples of ambient sound (black line) and boat noise (red dashed line). The dB SPL of ambient and noise samples within the hearing range of toadfish (20–800 Hz, Yan et al., 2000) were compared at Eel Pond (B) and Pier 40 (D). **** = p < 0.0001.

levels of boat traffic and compared this to a well-studied population of oyster toadfish located in an area of low boat traffic (Eel Pond, MA). We identified both commercial and recreational boats crossing our field site at Pier 40 (New York, NY), with an average of 35 boats per hour, imposing a significantly noisy environment on the animals in the area. Additionally, we assessed the calling behavior and the amount of noise at this location and compared it to the calling behavior in a quieter environment (Eel Pond, MA). At Pier 40, we found an inverse relationship between calling behavior and time in noise. We then conducted a microanalvsis to see if animals adjust their calling behavior during acute exposures to noise and did not observe any changes after short duration exposures (around 148 s). We found that toadfish at Pier 40 concentrated their calling behavior later in the night to potentially account for the constant noise

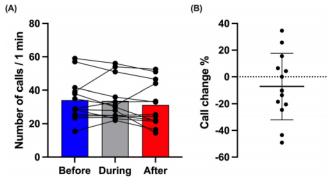


FIG. 9. (Color online) Oyster toadfish calls behavior before, during, and after acute noise exposure. (A) Calls were identified before (blue), during (gray), and after (red) exposure. Individual noise events where calls were counted are identified by circles with adjoining lines. (B) The call change % was calculated to assess the distribution of calling behavior; the mean and standard deviation are indicated by the bars. We saw an overall call change % average of –7.17, indicating a non-significant decrease in overall call behavior. No difference in call behavior is indicated by the dotted line (at 0).

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exposures they experience during daytime hours. Overall, the ambient acoustic scene at Pier 40 is significantly more intense than the acoustic scene at Eel Pond within the hearing range of oyster toadfish. Taken together, these results could indicate that oyster toadfish may be adjusting their call patterning over the course of an entire day to reduce possible masking and to potentially increase the likelihood of attracting a mate.

A. Characterization of boat noise near Pier 40, Hudson River

One goal of this study was to quantify boat activity and noise at our recording site on the Hudson River, Pier 40. A previous study examining the Hudson River documented biological sounds (chewing or unidentifiable fish sounds), and nonbiological sounds (boats and ferries), and identified four species of soniferous fish (Opsanus tau, Ophidion marginatum, Ameiurus nebulosus, and Ictalurus punctatus; Anderson et al., 2008). Most of the analyses were conducted between the hours of 21:00 and 6:00 because fish tend to vocalize most during the evening. The present study is the first of its kind to assess the types of boat activity and duration of anthropogenic noise in the lower Hudson River over a full day. We identified both commercial and recreational activities that passed Pier 40, up to 48 times an hour, including a state-regulated ferry that passes Pier 40 six times an hour. Additionally, boat activity would overlap to create a noisy acoustic scene up to 20 min.

B. Calling behavior varied between locations

After examining calls and noise over a three-day period, we found that toadfish call throughout the day and night, similar to other studies (Fine, 1977; Ricci et al., 2017; Van Wert and Mensinger, 2019). Toadfish at Pier 40 showed an inverse relationship between calling behavior and the proportion of noise, a potential adaptive mechanism to avoid masking their advertisement call for potential female mates. To analyze this further, we binned the calls at our two locations into four equal periods throughout the day. Toadfish at Pier 40 exhibit peak calling behavior between midnight and 5 AM (45.8% of daily calls), while toadfish at Eel Pond exhibit peak calling behavior between 6PM and midnight (51.9% of daily calls). This suggests that the toadfish at Pier 40 are calling the highest in the morning between 00:00 and 05:00 h, rather than peaking after sunset like the toadfish in Eel Pond and other non-urban locations which has been documented in previous studies (Fine, 1977; Ricci et al., 2017; Van Wert and Mensinger, 2019).

Additionally, we found that the ambient conditions of Pier 40 are significantly higher in dB SPL than the ambient conditions at Eel Pond, and are within the hearing range of oyster toadfish (Yan et al., 2000). Similarly, the noise present at Pier 40 is also higher in SPL than the ambient conditions at both Pier 40 and Eel Pond. Therefore, calling later might be a way to optimize their energy to produce calls during a time when they are less likely to experience noise

interference during high peaks of human activity, like boat traffic. However, it is important to note that other factors (salinity, chorusing, density of animals), not observed here, could also contribute to changes in calling behavior.

C. Acute noise events and calling behavior

Previous studies on toadfishes have found that exposure to intense noise can cause a decrease in hearing sensitivity, calling behavior, fecundity, number of fertilized eggs, and larval size (Vasconcelos et al., 2012; Alves et al., 2016; Krahforst et al., 2016; Rogers et al., 2020; Alves et al., 2021; Amorim et al., 2022). These studies utilized speakers to conduct experimental playback experiments, introducing artificial noise into the environment. The present study sought to correlate changes in toadfish calling behavior with anthropogenic noise events already present in their natural environment. To our knowledge, Mackiewicz et al. (2021) is the first study to examine the effects of in situ noise on call behavior of oyster toadfish and reported a decrease in calling behavior after exposure to an idling research vessel adjacent to the recording site in Eel Pond (the same location in the present study). In the present study, we did not identify significant behavioral changes during or after acute noise events but found an average overall decrease in call rate (-7.1%).

Additionally, given the research vessel's proximity to the hydrophones and the toadfish at Eel Pond (as close as 5 m), they were unable to count toadfish calls during exposure because the noise was too intense (up to 126 dB) and too low in frequency (100-500 Hz) to properly assess toadfish calls (Mackiewicz et al., 2021). The distance of our hydrophone location at Pier 40 to the center of the Hudson River is about 350 m away. Vessels pass through the Hudson River at varying distances from Pier 40, which could range from 245 to 350 m from our location. The fish at Pier 40 are exposed to passing vessels up to 48 times an hour and are detectable by the hydrophone. However, the amplitude of frequencies of noise in the hearing range of toadfish might not be intense enough to reach a threshold that induces an acute change in calling behavior. While noise exposures captured in Mackiewicz et al. (2021) from Eel Pond may be more infrequent, they could be more intense given the proximity of the vessel to the fish.

D. Limitations

Our study examines oyster toadfish calling behavior in relation to anthropogenic noise over the course of three consecutive 24-h days at Pier 40, compared to two days in May, and single day in June at Eel Pond. While it is not ideal to compare populations at two different sites on different days, our goal was to examine the behavior based on temperature since toadfish calling behavior changes dependent on water temperature (Edds-Walton *et al.*, 2002; Maruska and Mensinger, 2009; Tavolga, 1958; Van Wert and Mensinger, 2019). For this reason, we selected acoustic recordings at Eel Pond where the temperatures of the data collected at



Pier 40 was matched (between 17 °C and 18 °C). However, we noticed a significant difference in calls between May 27th and 28th and June 6th, only ten days apart. Despite a change in the number of calls at Eel Pond, the daily pattern of calls between these dates remains the same.

While hourly salinity was not measured in this study, our two locations receive different sources of water with varying salinity. Pier 40 is part of the Hudson River estuary where there is a circulation of both fresh and salt water that changes depending on the tide while Eel Pond contains only sea water. However, we do not believe that the salinity of the water is affecting the daily calling behavior of the oyster toadfish at Pier 40 since the general diel patterns match previous studies (Fine, 1977; Ricci *et al.*, 2017; Van Wert and Mensinger, 2019).

Previous studies on Bocon toadfish (*Amphichthys cryptocentrus*) identified that the density and distribution of toadfish can impact the characteristics of the toadfish chorus (Salas *et al.*, 2018). Additionally, the authors predicted that an increase in calling behavior by nearby conspecifics may stimulate other males who are within the same active state. However, we cannot identify how many animals were present at our two field locations. For this reason, we measured the proportion of daily calling patterns to compare our locations more directly.

E. Future studies

A long-term PAM experiment across multiple locations within the Hudson River system is necessary to see if there are behavioral changes at different parts of the reproductive season or based on the level of normal boat activity. Gulf toadfish (*Opsanus beta*) have been proposed as an indicator species of estuary conditions, since their calling behavior changes as a result of salinity (Fournet *et al.*, 2019), therefore measuring hourly salinity might provide additional information on the call patterns throughout a day or season.

A multi-hydrophone array could provide more information about the number of animals present at each location and other acoustic information such as amplitude that would be important in determining which noise events specifically fall within the hearing sensitivity range of *O. tau*. Previous research on oyster toadfish have shown that there are individual differences in calling rate, amplitude, waveform, fundamental frequency, and duration (Edds-Walton *et al.*, 2002; Amorim and Vasconcelos, 2008; Putland *et al.*, 2018), therefore, monitoring changes of individual calling behavior would provide more insight into the individual effects of noise on an animal. Additionally, a study examining calling behavior and boat noise patterns throughout a longer period of time at both locations would confirm these results.

Overall, the present study has begun to document boat noise contributing to the soundscape of the Hudson River at Pier 40 where soniferous fish, like oyster toadfish, are abundant, and examined diel patterns of calling behavior in relation to the abundance of anthropogenic noise. In contrast to an environment with minimal boat traffic (Eel Pond), oyster toadfish living in a major urban setting may be adjusting their peak calling later through the early am hours, potentially to avoid this essential reproductive signal from being masked by noise during hours of greater human activity. The study location at Pier 40 provided the opportunity to observe possible acclimations of toadfish to thrive in a soundscape that has been strongly impacted by anthropogenic noise.

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